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## ELECTRONIC LETTER

Mutations in *FLNB* cause boomerang dysplasia

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Boomerang dysplasia (BD) is a perinatal lethal osteochondrodysplasia, characterised by absence or underossification of the limb bones and vertebrae. The BD phenotype is similar to a group of disorders including atelosteogenesis I, atelosteogenesis III, and dominantly inherited Larsen syndrome that we have recently shown to be associated with mutations in *FLNB*, the gene encoding the actin binding cytoskeletal protein, filamin B. We report the identification of mutations in *FLNB* in two unrelated individuals with boomerang dysplasia. The resultant substitutions, L171R and S235P, lie within the calponin homology 2 region of the actin binding domain of filamin B and occur at sites that are evolutionarily well conserved. These findings expand the phenotypic spectrum resulting from mutations in *FLNB* and underline the central role this protein plays during skeletogenesis in humans.

**B**oomerang dysplasia (OMIM #112310) is a lethal skeletal dysplasia characterised by disorganised skeletal ossification.<sup>1</sup> Characteristic radiological findings include irregular ossification of the limb bones and vertebrae, underdevelopment of the acetabulum, and hypoplastic, boomerang shaped femora from which the disorder derives its name.<sup>1</sup> At least eight cases have been described in the literature to date.<sup>1-8</sup> A similar lethal skeletal dysplasia, Piepkorn dysplasia,<sup>9</sup> has been proposed to be allelic to boomerang dysplasia.<sup>2,7</sup> Cartilage histology demonstrates both disorganised cartilage anlagen of the developing long bone and frequent multinucleated chondrocytes in the reserve zone of the growth plate.<sup>1</sup> Other developmental abnormalities beyond the skeleton have been described, including omphalocele and encephalocele.

Similarities in the radiographic and histological findings of boomerang dysplasia and atelosteogenesis I (AOI; OMIM #108720) and III (AOIII; OMIM #108721) have suggested allelism for these disorders.<sup>2,10</sup> In all three entities, radiographic findings include deficient and irregular ossification of bones and vertebral bodies with distal humeral hypoplasia and joint dislocations in addition to craniofacial abnormalities.<sup>11</sup> Additionally, multinucleated chondrocytes have been observed in both AOI and boomerang dysplasia. Boomerang dysplasia is distinguished from AO on the basis of a more severe defect in mineralisation, with complete absence of ossification in some limb elements and vertebral segments.<sup>3</sup> A preponderance of males among reported cases of boomerang dysplasia<sup>7</sup> and some phenotypic similarities to the otopalatodigital syndrome spectrum disorders caused by mutations in the *FLNA* gene<sup>12,13</sup> suggested an X linked aetiology to some authors.<sup>7</sup>

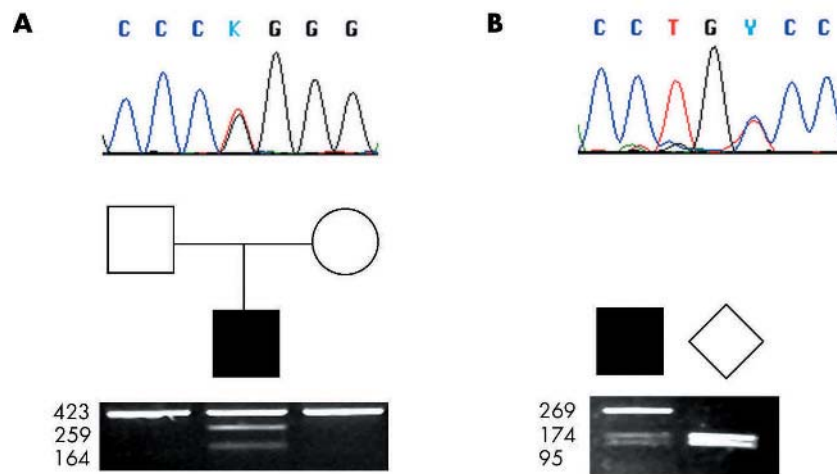
Recently we described the association of mutations in the gene encoding the 280 kDa cytoskeletal protein filamin B with several disorders characterised by skeletal defects and joint dislocations.<sup>14</sup> Filamin B is a modular protein. An actin

binding domain composed of two calponin homology domains (CH1 and CH2) is located at the amino terminus, followed by a rod domain composed of 24 structurally homologous repeats and terminating in a carboxyterminal dimerisation domain.<sup>15</sup> The actin binding domain in filamin B is similar to those found in other cytoskeletal proteins, such as  $\beta$ -spectrin, dystrophin, and  $\alpha$ -actinin-4.

Four distinct conditions have been attributed to mutations in *FLNB*: AOI, AOIII, recessively inherited spondylcarpotarsal syndrome (OMIM #272460), and a dominant form of Larsen syndrome (OMIM #150250). Individuals with AOI, AOIII, and dominantly inherited Larsen syndrome were found to be heterozygous for missense mutations in *FLNB*.<sup>14</sup> The majority



**Figure 1** Radiograph of case 1. The calvarium, spine, and pubis are underossified with a thoracic scoliosis evident. There is severe micromelia with unossified humeri, absent femora, and severely bowed and shortened radii, ulnae, and tibiae. No ossification is seen in the hands or feet.



**Figure 2** Chromatograms and restriction endonuclease digestions demonstrating mutations associated with boomerang dysplasia. (A) Case 1, showing heterozygosity for the mutation 512T→G and restriction endonuclease digestion using *HpaII*. Analysis of the parents (lanes 1 and 3) is shown alongside that of the proband (lane 2). (B) Case 2, showing heterozygosity for the mutation 703T→C, and restriction endonuclease digestion by *DrdI*. Analysis of amplified DNA from the proband (lane 1) and a healthy, unrelated control (lane 2). Fragment sizes are indicated in bp.

of the mutations leading to AOI or AOIII lead to substitutions localised to the CH2 domain of the actin binding domain of filamin B. The sole exception is the mutation 2251G→C, located in exon 15, which is predicted to lead to the substitution G751R in repeat 6, found in an individual with AOIII.<sup>14</sup> During the work that identified the group of disorders resulting from mutations in *FLNB*, one individual with boomerang dysplasia was assessed for mutations by direct sequencing, but no mutation was identified.<sup>14</sup>

Given the radiographic, phenotypic, and histological evidence suggesting that boomerang dysplasia is related to disorders caused by mutations in genes encoding filamins, we examined two individuals with boomerang dysplasia for mutations in *FLNA* and *FLNB* using denaturing high performance liquid chromatography (DHPLC) analysis as the screening technique.

Individual 1 was the case previously examined by direct sequencing. This male fetus was the first child of non-consanguineous, healthy parents. Second trimester ultrasound scanning revealed thoracic hypoplasia and marked shortening of all limbs with irregular ossification of multiple skeletal elements. The fetus was delivered at 22 weeks' gestation. Post-mortem analysis demonstrated marked thoracic hypoplasia, short limbs, and the characteristic facial dysmorphism associated with boomerang dysplasia. There was no encephalocele or omphalocele present. The radiographic findings were typical for boomerang dysplasia (fig 1).

Individual 2 was a fetus of 17 weeks' gestation, previously reported in detail by Wessels *et al.*<sup>8</sup> This fetus demonstrated severe micromelia with two of the three long tubular bones missing in all four limbs. On radiographic examination, the remaining radii were boomerang shaped, the tibiae were segment-like, the spine was poorly ossified, and the thorax was hypoplastic. Histologically, the cartilage matrix was hypocellular with multinucleated giant chondrocytes present.

The coding regions and intron-exon splice junctions of both *FLNA* and *FLNB* were amplified from genomic DNA obtained from both cases using intronic primers described previously<sup>13 14</sup> (reaction conditions and primer sequences are available on request). For analysis of *FLNA*, amplicon products were visualised by agarose gel electrophoresis and then mixed with equal amounts of PCR amplified products from an unrelated healthy male control before heteroduplex formation. Samples were subjected to DHPLC on a WAVE DNA fragment analysis system (Transgenomic Inc.) according to the manufacturer's instructions. Amplicons

demonstrating anomalous trace forms were re-amplified and cycle sequenced on an ABI 3100 sequencer. Identified mutations were confirmed by restriction enzyme digest of amplified DNA, and parentage was confirmed as declared for case 1 by the segregation of six unlinked microsatellite markers. Parental samples were unavailable for case 2. This study was approved by the Otago ethics committee.

Examination of *FLNB* (exons 1–46) revealed mutations in both subjects. In case 1, the individual was shown to be heterozygous for the mutation 512T→G, which is predicted to lead to the substitution L171R. This mutation creates a site for the restriction enzyme *HpaII*, enabling the demonstration that it had arisen de novo in this family (fig 2), providing genetic evidence of pathogenicity. In retrospect, this mutation was present in the original analysis, but it was overlooked. The second mutation, 703T→C, was found in exon 4 in case 2, and predicts the substitution S235P (fig 2). Parental samples were not available, but this mutation was not present in 100 control chromosomes. Both mutations are predicted to alter residues within the CH2 region of the actin binding domain of filamin B. These residues are highly evolutionarily conserved among vertebrate filamins (fig 3). The mutations suggest that these amino acids play a critical role in actin binding domain function. For the 703T→C mutation, the same substitution has been observed at the analogous residue in *ACTN4*, a gene that encodes the cytoskeletal adapter protein  $\alpha$ -actinin-4 (fig 3), in a patient with focal and segmental glomerulosclerosis.<sup>16</sup>

Disease associated substitutions within the actin binding domain of filamin B lead to a diversity of phenotypes. Previously we have reported substitutions leading to AOI ( $n = 3$ ), AOIII ( $n = 1$ ), and Larsen syndrome ( $n = 2$ ).<sup>14</sup> All of these phenotypes are associated with defects in skeletogenesis and joint formation. The identification of mutations leading to substitutions within the actin binding domain of filamin B in two patients with boomerang dysplasia confirms that this condition is allelic to these disorders reflecting previous clinical suspicions of relatedness.<sup>2 3</sup>

A proportion of mutations that cause the otopalatodigital spectrum of skeletal disorders lies within the analogous CH2 region in *FLNA*. These mutations also lead to a broad spectrum of phenotypic severity.<sup>13</sup> There is no simple correlation between the location of these mutations and the severity of the resultant defects in skeletal ossification. While otopalatodigital syndrome type 1 manifests more mildly than otopalatodigital syndrome type 2, these conditions can result

<b>A</b>	Filamin A Human	PQLP---ITNFSRDWQSGRALGALVDSCAPGL	209
	Filamin B Human	PYLP---ITNFNQNWQDGKALGALVDSCAPGL	182
	Filamin C Human	PQLP---ITNFNRDWQDGKALGALVDNCAAPGL	202
	$\alpha$ -Actinin4 Human	APYKNVNVQNFHISWKDGLAFNALIHRHPEL	209
	$\beta$ -spectrin Human	AGYPHVNVITNFTSSWKDGLAFNALIHKHRPDL	219
	Filamin B Mouse	PYLP---ITNFNQNWQDGKALGALVDSCAPGL	182
	Fln1 D. melanogaster	PDLF---INNFTNDWTTGKAVGALVDACAPGL	315
	Hyp-Fln A. gambiae	PDLF---INNFTSDWTNGKAVGALVDAVAPGL	189
	ABP-120 D.discoideum	APYKV-VNNFTDSWCDGRVLSALTDSLKPGLV	170
	Filamin C.elegans	PELK---LTNFRNTNWDGIALSALLEYCPGL	168

<b>B</b>	Filamin A Human	LGIPQVITPEE-IVDPNVDEHSVMTYLSQFPK	266
	Filamin B Human	LGVPQVITPEE-IIHPDVDEHSVMTYLSQFPK	239
	Filamin C Human	LGVPQVIAPEE-IVDPNVDEHSVMTYLSQFPK	259
	$\alpha$ -Actinin4 Human	LDIPKMLDAEDIVNTARPDEKAIMTYVSSFYH	266
	$\beta$ -spectrin Human	LGIIPLLDPED-VFTENPDEKSIITYVVAFYH	275
	Filamin B Mouse	LGVPQVITPEE-IIHPDVDEHSVMTYLSQFPK	239
	Fln1 D. melanogaster	LNVRQLIKPEE-LVNPVDEQSMMTYLSQYPN	372
	Hyp-Fln A. gambiae	LNVRQLIRPEE-MVNPENIDEQSMMTYLSQYPN	246
	ABP-120 D.discoideum	YEIPKIMDAN--DMNSLPDELSVITYVSYFRD	224
	Filamin C.elegans	LEVPPITSSDH-LSSPHLDELSCLTLYSYFIT	225

**Figure 3** ClustalW alignment of filamin and proteins with actin binding domains from human, mouse, *Drosophila melanogaster*, *Anopheles gambiae*, *Dictyostelium discoideum*, and *Caenorhabditis elegans*. (A) Sequence contexts around residues L171; (B) S235. Hyp-Fln, hypothetical filamin.

from substitutions at residues in close proximity to one another or even at the same residue.<sup>13–17</sup> The observation that mutations in the actin binding domain of *FLNB* can also lead to a wide spectrum of severity recapitulates this pattern. The substitution predicted by the mutation found in individual 1, L171R, is in close proximity to the site of a substitution (A173V), causative for AOI.<sup>14</sup> Similarly, the S235P substitution reported here is within the same protein fold as E227K, a substitution associated with the less severe, non-lethal phenotype, Larsen syndrome.<sup>14</sup> Despite reports of disease associated substitutions in residues within the CH2 of the actin binding domain of dystrophin,  $\alpha$ -actinin-4,<sup>17</sup> filamin A,<sup>13</sup> and filamin B,<sup>14</sup> a functional role for this protein fold has not been definitively elucidated. As CH2 domains do not possess any innate actin binding properties in isolation, it has been postulated that the role of the CH2 in actin binding is, at best, an indirect one.<sup>18</sup>

Filamins act primarily to stabilise actin within the cell, either as parallel bundles or orthogonal gel networks.<sup>19–20</sup> Multiple binding partners have been identified for filamin A, the vast majority associating through filamin repeats 16–24 (reviewed in Stossel *et al*<sup>21</sup>). Five binding partners have been shown to associate with filamin B: actin, FBLP-1,<sup>22</sup> presenilin (PS1 and PS2),<sup>23</sup> and GPIIb.<sup>24</sup> It remains to be seen whether mutations within the actin binding domains of filamins A and B exert their pathogenic effect primarily by disturbing actin binding or through a functional dependency between actin association and binding interactions with other proteins.

Multinucleated giant cells are found among chondrocytes in the growth plate of individuals with boomerang dysplasia. These appearances could result from defective cell cleavage during the proliferation of chondrocytes within the epiphyseal growth plate. Previously we have demonstrated widespread expression of *FLNB* within the growth plate and most notably in the cleavage furrow of dividing chondrocytes.<sup>14</sup> The process of altered cell cleavage leading to both the defective skeletal patterning and mineralisation in the boomerang dysplasia/atelosteogenesis group of disorders remains to be elucidated.

The identification of mutations causing boomerang dysplasia in *FLNB* confirms boomerang dysplasia as part of the chondrodysplasia spectrum including AOI and III, and

non-lethal Larsen syndrome. Phenotypic overlap between boomerang dysplasia and AO, as reported here and previously, and the colocalisation of mutations underlying these diseases in exons encoding the actin binding domain of *FLNB* is indicative of a clinicopathological spectrum for these disorders. This further broadening of the phenotypic spectrum associated with mutations in *FLNB* highlights the importance of a functional actin cytoskeleton for many normal morphogenetic processes, including skeletogenesis.

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